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Chemical and Physical Defense of Weed Seeds in Relation to Soil Seedbank Persistence

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Effective weed seedbank management requires mechanistic understanding of ecological determinants of seed persistence in the soil seedbank. Chemical and physical defense of common lambsquarters, field pennycress, giant foxtail, kochia, velvetleaf, and yellow foxtail seeds were quantified in relation to short- and long-term seedbank persistence. Seed content of *ortho*-dihydroxyphenols (*o*-DHP), a class of putative seed defense compounds, varied more than threefold between the least protected species (common lambsquarters, 9.2 μg g seed^{-1}) and the most protected species (kochia, 34.1 μg g seed^{-1}). Seed *o*-DHP was inversely related ($r = -0.77$, $P < 0.001$) to seed half-life in the soil and to short-term seed persistence in burial assays ($r = -0.82$, $P < 0.05$). The relative importance of chemical seed protection in comparison to physical seed protection, as represented by the ratio of seed *o*-DHP concentration to seed coat thickness, decreased linearly with increasing short-term seed persistence ($r = -0.96$, $P < 0.01$) and nonlinearly with increasing long-term seed persistence in the soil seedbank ($y = 0.16 + 0.21/(0.0432 + x)$, $R^2 = 0.99$, $P < 0.001$). Mechanical damage to the seed coat, via piercing, slicing, or grinding treatments, increased short-term mortality during burial for all six species. Mortality of pierced seeds was negatively associated ($r = -0.35$, $P < 0.05$) with seed phenol concentration and positively associated with seed half-life ($r = 0.42$, $P < 0.01$) and seed coat thickness ($r = 0.36$, $P < 0.05$). Seed phenolics, as a class, supported the results for *o*-DHPs. Overall, these findings suggest a potential weakness, with respect to seedbank management, in the way weed seed defenses are constructed. Weed species with transient seedbanks appear to invest more in chemical defense than those species with highly persistent seedbanks. As a result, seeds in the latter category are relatively more dependent upon physical seed protection for persistence in the soil seedbank, and more vulnerable to management tactics that reduce the physical integrity of the weed seed coat.

Nomenclature: Common lambsquarters, *Chenopodium album* L.; field pennycress, *Thlaspi arvense* L.; giant foxtail, *Setaria faberi* Herrm.; kochia, *Kochia scoparia* (L.) Schrad.; velvetleaf, *Abutilon theophrasti* Medik.; yellow foxtail, *Setaria glauca* (L.) Beauv.

Key words: Seed coat, physical protection, mechanical damage, *ortho*-dihydroxyphenols, phenolic compounds, seed longevity, decay, half-life.

Soil seedbanks are comprised of both dormant and nondormant seeds persisting at varying depths within the surface soil profile. As such, they are an adaptive mechanism by which plants sample variable environments across time (Fenner and Thompson 2005; Froud-Williams 1987). For annual weed species of arable fields, the formation of persistent seed pools is particularly important because features of the agricultural environment, such as soil disturbance and high nutrient availability, can function as germination cues (Benech-Arnold et al. 2000). If all seeds within a weed seedbank were to germinate within a given year, weed management would be a simple matter, with one or two fallow years permanently eradicating seeds from a field. Instead, the seeds of most annual weed species are capable of persisting in the soil seedbank for several years or more (Buhler and Hartzler 2001; Burnside et al. 1996; Conn et al. 2006; Lueschen et al. 1993; Roberts and Feast 1972), and thus reinfesting arable fields for many years after a single seed rain event. Due to the demographic centrality of the seed life stage for annual weeds, reducing the persistence of seeds in the soil seedbank is a critical goal for long-term management of weed populations, yet this topic has received relatively little scientific attention to date (Buhler 2002; Davis 2006; Wagner and Mitschunas 2008). Thus, there is a pressing need for increased mechanistic understanding of factors controlling seed longevity in the soil seedbank.

Theoretical components of seedbank persistence for a given cohort of seeds include seed losses due to germination, aging, emigration, and mortality (Fenner and Thompson 2005; Schafer and Chilcote 1969). Most viable weed seeds within persistent soil seedbanks are found beneath the soil surface but within the top 30 cm of the soil profile (Harrison et al., 2007; Reuss et al. 2001). Postdispersal seed predation (Heggenstaller et al. 2006; Menalled et al. 2006) causes much of the weed seed rain in arable fields to disappear from the soil surface within days to weeks. Remaining seeds can find their way into deeper layers in the soil profile through various processes, including soil disturbance (Yenish et al. 1992), seed caching by granivores (Hartke et al. 1998; Pemberton and Irving 1990), or seed movement into soil cracks (Thompson et al. 1993). Dormancy cycling helps to ensure that for most weed species only a small proportion of buried seeds is recruited as seedlings from the soil seedbank in any given year (Baskin and Baskin 2001). Those seeds that remain dormant in the soil seedbank must maintain their viability while withstanding attack from soil invertebrates and microbes (Chee-Sanford et al. 2006).

Seeds also have inherent characteristics that determine their persistence in the soil seedbank. The ageing rate of seeds determines their maximum longevity in soil seedbanks and varies among species (Telewski and Zeevaart 2002; Toole and Brown 1946) and maternal environments (Donald 1993). Within this theoretical maximum, seeds maintain viability and structural integrity through chemical and physical protection mechanisms. A wide variety of secondary metabolites, such as phenols and alkaloids, can be concentrated in

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seed components for defense purposes (Halloin 1983; Hendry et al. 1994; Kremer 1993; Veldman et al. 2007). Variation in seed coat strength appears to confer differential protection from seed predation; seeds of species adapted for ant dispersal possess stronger seed coats and are more likely to end up in seed caches than those with weaker seed coats that are simply eaten by ants (Rodgerson 1998). Hard-seededness in weed species such as common lambsquarters and velvetleaf helps deter fungi from penetrating the seed (Kremer et al. 1984), but does not prevent substantial seed losses of these species due to postdispersal predation (Cardina et al. 1996; Carmona et al. 1999; Westerman et al. 2006).

This study examines the contribution of chemical and physical seed defenses to seedbank persistence for six commonly occurring weed species of the midwest United States, including four forbs (common lambsquarters, field pennycress, kochia, and velvetleaf) and two grasses (giant foxtail and yellow foxtail) (Bridges and Baumann 1992). Study species were chosen to form a group representing a wide range in seedbank persistence (Buhler and Hartzler 2001; Burnside et al. 1996; Lueschen et al. 1993). We hypothesized that seed defenses are a primary driver of weed seedbank persistence; i.e., seed mortality imposes a fundamental limit on seed persistence in the soil seedbank, and better-protected seeds will therefore last longer in the soil (Kremer 1993; Menalled et al. 2006). Under this assumption, we expected that both chemical and physical protection of seeds would be positively associated with the half-life of buried seed pools of various weed species. To test this hypothesis, we determined seed concentrations of phenolic compounds, with special emphasis on *ortho*-dihydroxyphenols, which include a wide variety of defense compounds (Hendry et al. 1994). We also assayed the effect of varying severity of mechanical damage to the seed coat on seed mortality during a 2-mo burial in field soil. Data from these investigations were related to short-term weed survival in the burial assays and half-lives for seed persistence in the soil seedbank estimated for each of the study species from long-term seed burial studies in the midwest United States (Buhler et al. 2001; Burnside et al. 1996; Lueschen et al. 1993).

Materials and Methods

Seed Chemical Protection. Seed chemical defense levels, as represented by whole-seed concentrations of phenolic compounds, with specific emphasis on *ortho*-dihydroxyphenols (Hendry et al. 1994), were measured for common lambsquarters, field pennycress, giant foxtail, kochia, velvetleaf, and yellow foxtail. Phenols form a broad class of antiherbivore plant defense compounds, within which the *ortho*-dihydroxyphenols include simple and common phenols and phenolic acids (Harborne 1991; Hendry et al. 1994). Experimental seed lots were collected in fall of 2002 from ambient weed populations along margins of a field located at the Michigan State University Crop and Soil Science farm in East Lansing, MI, USA (42°42′27.72″N, 84°28′15.67″E). Mature seed heads were gently shaken to obtain ripe seed. Seed was bulked to form a composite accession and stored at 4 C in airtight plastic containers prior to initiation of the experiment.

Seed concentrations of phenols and *ortho*-dihydroxyphenols were determined in two ways. For determination of seed concentrations of *ortho*-dihydroxyphenols as a class, we used a colorimetric asssay, described in full detail in Hendry and Grime (1993). Briefly, a 2 g subsample of each experimental seed lot was ground to a fine homogenate in a benchtop precision mill.[1] Next, 60 mg of seed homogenate was extracted in 0.75 ml MeOH:0.75 ml 100 mM Tris-HCl buffer (pH 6.7) and centrifuged at 12,300 × *g* for 2 min. Methanol was used as an extractant in order to recover as much of the phenolic compounds in the seed homogenates as possible. Although this overrepresents what soil organisms would encounter in soil solution at any given point in time, it provides a better overall estimate of seed phenolic concentrations than aqueous extracts. A 250 µl sample of the supernatant was then added to 1 ml 100 mM KTi oxalate and 375 µl Tris-HCl buffer in a 2 ml plastic cuvette. The absorbance of the solution in the cuvette was then measured in a spectrophotometer[2] at a wavelength of 445 nm. Sample absorbances were converted into *ortho*-hydroxyphenol concentrations by means of a pyrogallol standard curve ranging from 0 to 1,000 nM in 100 nM increments. The procedure was repeated twice for each of the study species.

The remainder of the methanol seed extract for each species was brought to the Metabolomics Unit of the University of Illinois Roy Carver Biotechnology Center, in Urbana, IL, for gas chromatography–mass spectrometry (GC/MS) analysis, providing a full profile of phenolic compounds in the seeds, including *ortho*-dihydroxyphenols. Sample methanol extracts were divided into two parts, for analysis of underivatized and derivatized phenolic compounds (Shulaev 2006). First, a 100 µl aliquot of seed extract was set aside for analysis on a Zebron ZB-WAX *plus* column[3] (30 m, 0.25 mm ID, 0.25 µm film thickness). A 5 µl aliquot of the sample was injected in splitless mode to the GC/MS system, consisting of an Agilent 6890N GC, an Agilent 5973 mass selective detector, and HP 7683B autosampler.[4] The unit was programmed for the following operating conditions: warm-up from ambient temperature to 50 C for 5 min, followed by 12 C min^{-1} to 260 C, stabilizing at 260 C for 10 min. The injection port and transfer line temperatures were 280 C and 250 C, respectively. The helium carrier gas was set at a constant flow rate of 1.5 ml min^{-1}. Mass spectra were recorded in the m/z 50–800 scanning range.

A second aliquot of the methanol seed extract (~400 µl of each extract) was re-extracted with dimethyl ether, dried, and derivatized with MSTFA (N-Methyl-N-(trimethylsilyl) trifluoroacetamide) for 1 hr at 60 C to increase volatility of the phenolic compounds contained in the sample (Shulaev 2006). This step is necessary to detect those phenolic compounds that would otherwise not be volatile enough to enter the gas chromatograph under the operating conditions described above. Sample volumes of 5 µl were injected with a splitless mode to the same GC/MS system. Injections were performed on a 30 m HP-5MS column[4] with 0.25 mm ID and 0.25 µm film thickness with an injection temperature of 250 C, the interface set to 250 C, and the ion source adjusted to 230 C. The He carrier gas was set at a constant flow rate of 1.3 ml min^{-1}. The temperature program was 5 min isothermal heating at 70 C, followed by an oven temperature increase of 5 C min^{-1} to 310 C and a final 10 min at 310 C. Mass spectra were recorded in the m/z 50–800 scanning range.

The spectra of all chromatogram peaks were compared with two electron impact mass spectra libraries: NIST05 and the Wiley Registry, 8th Edition.[5] To allow comparison between

samples, all data were normalized to the internal standard (cinnamyl alcohol 1 mg ml^{-1}) in each chromatogram and weight of each sample. The chromatograms and mass spectra were evaluated using the HP Chemstation[4] and AMDIS[6] programs.

Seed Physical Protection. Quantitative differences in seed physical protection among species were represented by measurements of seed coat thickness with a field-emission environmental scanning electron microscope[7] maintained by the Imaging Technology Group of the University of Illinois Beckman Institute. Seeds of each species were embedded in paraffin, sliced in half with a scalpel, and sputter-coated[8] with a nanolayer of Au/Pd. Thickness of the inner and outer integuments of prepared seeds was measured using image analysis software.[9]

Mechanical Damage Bioassays. We examined the effect of mechanical damage to the weed seed coat on seed mortality in the soil seedbank through controlled-environment bioassays. The treatment design consisted of a factorial combination of four intensities of damage to the seed coat applied to the seeds of the six study species, which were then incubated in field soil for 2 mo. The experiment was conducted in a randomized complete block design with four replications and repeated in two sequential experimental runs.

Field soil for bioassays was collected in late March 2003, to a depth of 10 cm from an area of the Long Term Ecological Research Site (Davis et al. 2005) at the Michigan State University W. K. Kellogg Biological Station in Hickory Corners, MI. This field was managed in an early successional state through annual burning and mowing. The soil collected from this site was a Kalamazoo silt loam (Fine-loamy, mixed, semiactive, mesic Typic Hapludalfs; 43% sand, 40% silt, 17% clay, 1.1% organic carbon, and pH 6.7). Soil samples were bulked to form composite samples and stored in sealed plastic bags at 4 C prior to use. Subsamples of the study soil were processed in a mechanical elutriator (Wiles et al. 1996) to determine the ambient population density of seeds of study species. This procedure indicated that there were no viable seeds of any of the six species in the study soil; therefore, ambient population density of these seeds was not included in seed mortality calculations for the bioassay.

Four types of damage treatments were applied to weed seed coats: no damage, piercing, slicing, and grinding (Figure 1). Under a dissecting microscope, seed coats were pierced with a sterile 0.6 mm diameter needle (sharpened point of needle was < 0.1 mm) in an area of the seed distal to the embryo axis. In the slicing treatment, seed coats were sliced with a sterile scalpel in an area distal to the embryo axis, creating a 1-mm-diam opening. The fourth damage class, grinding, was achieved by subjecting batches of 40 seeds to a 1 s pulse in a coffee grinder.[10]

Prior to incubations, seeds from the various damage treatments were subjected to tetrazolium testing (AOSA 2000) to determine initial seed lot viability. Bioassay experimental units consisted of 40 seeds of a given species buried at a 5 cm depth in field soil contained in a plastic cup 5 cm in diameter and 10 cm deep. Experimental units were incubated[11] for 2 mo at a constant temperature of 15 C, with 120 μmol PAR supplied during a 12 hr photoperiod. This relatively cool incubation temperature was chosen to simulate late fall/early spring conditions in the north central United States, when little seedling emergence is taking place, especially for summer annual species which comprised the majority in this study. Demographic models predict that seedbank persistence during this overwinter period should have the greatest effect upon population dynamics of annual weeds (Davis 2006). Initial gravimetric soil moisture was 19%, corresponding to a matric potential of −33 kPa, or approximately field capacity. The initial weight of each experimental unit was recorded, and all units were subsequently weighed on a twice weekly basis, with additional water added to make up the mass deficit at each weighing. Emerged seedlings were counted and removed weekly. At the end of the 2 mo incubation period, seeds were recovered from the soil in each experimental unit via elutriation and the number of viable seeds remaining was quantified with tetrazolium testing.

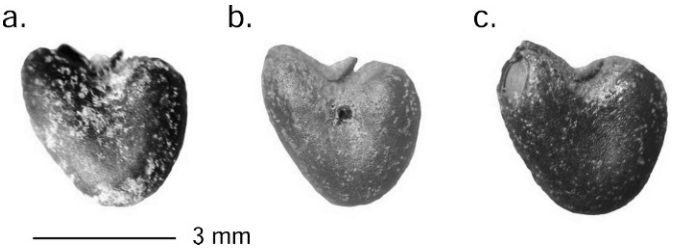

Figure 1. Seed coat mechanical damage classes (velvetleaf seeds shown here) included (a) no damage, (b) piercing, (c) slicing, and grinding (not shown).

Seedbank Persistence. Half-lives of seeds of the six study species in the soil seedbank were estimated by reanalyzing literature data obtained from field studies of seed burial lasting at least 5 yr and located in the midwest United States (Buhler and Hartzler 2001; Burnside et al. 1996; Lueschen et al. 1993). In each case, seeds were buried in untilled soil typical of that used in commercial corn (*Zea mays* L.) production, and annual seedling emergence from these seed pools was recorded. Nonlinear least-squares regression was used to relate the proportion of the initial seed pool emerging as seedlings to seed burial time for each of these species. Estimation of $t_{0.5}$ in this study was based on an assumption of constant decay proportions acting across years, giving rise to exponential decline in seedbank numbers (Conn et al. 2006). A negative exponential function of the form $y = ae^{-kt}$, where a represents initial seedbank viability and k is the exponential decay constant, was fit to the data using the NONLIN subroutine of SYSTAT 11.0.[12] Seed half-life in the soil seedbank ($t_{0.5}$) for each of these species was calculated as $t_{0.5} = \log_e(0.5)/-k$.

Statistical Analysis. Seed mortality during the 2 mo buried incubation in soil was calculated as $\mu = (s_0 - s_1 - g)/s_0$, where s_0 = the number of viable seeds added at the start of the bioassay, s_1 = the number of viable seeds recovered at the end of the assay, and g = the number of emerged seedlings. Seed mortality data were $\sin^{-1}(x^{0.5})$-transformed to meet ANOVA assumptions of normality and constant error variance (Neter et al. 1996). Within species, means of transformed data were separated by damage type based on a protected Bonferroni-corrected multiple comparison test (Neter et al. 1996). Back-transformed data were presented for simplicity of interpretation. Seed mortality associated with different mechanical

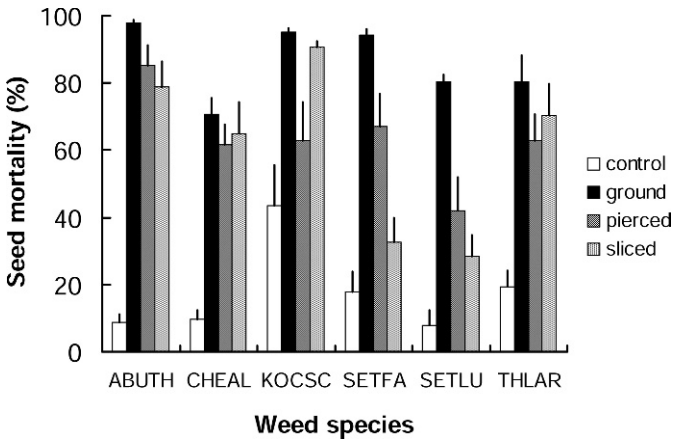

Figure 2. Percent mortality of seeds subjected to varying intensities of mechanical damage and incubated in field soil for 2 mo. Bars represent the mean of two runs of four experimental replicates, with standard errors of the mean. Explanation of Bayer codes: ABUTH = velvetleaf, CHEAL = common lambsquarters, KOCSC = Kochia scoparia, SETFA = giant foxtail, SETLU = yellow foxtail, THLAR = field pennycress.

damage treatments was determined by subtracting the base mortality level of undamaged seeds for a given species from seed mortality for a particular damage treatment.

Functional relationships between measures of chemical and physical seed protection and $t_{0.5}$ were examined using nonlinear least-squares regression. A negative exponential function was fit to chemical protection data, with seed phenol content as the dependent variable and $t_{0.5}$ as the independent variable, using the NONLIN subroutine of SYSTAT 11.0.[12] Seed physical protection data were fit to a natural logarithm function that included a y-intercept parameter, with seed coat thickness as the dependent variable and $t_{0.5}$ as the independent variable. Finally, the ratio of seed phenol content to seed coat thickness was related to variation in seed half-life via three parameter hyperbolic function, $y = y_0 + (b_1 \cdot b_2)/(b_2 + x)$, where the chemical:physical protection ratio was the dependent variable and $t_{0.5}$ was the independent variable.

Results and Discussion

Seedbank Persistence. Mortality of undamaged seeds during the 2-mo incubation in field soil varied from less than 10% for common lambsquarters, velvetleaf and yellow foxtail to over 40% for kochia (Figure 2). Short-term mortality of undamaged seeds during the incubations decreased exponentially in relation to increases in estimated $t_{0.5}$ for the study species ($\mu = 11.4 + 46.9e^{-2.5t_{0.5}}$, $R^2 = 0.91$, $P < 0.05$). Estimated seedbank persistence varied greatly for the study species, with values of $t_{0.5}$ varying from < 1 yr for giant foxtail and kochia to > 20 yr for common lambsquarters (Table 1). When these results were extrapolated out to the time of 99% seedbank depletion, species fell into three categories corresponding to the seedbank classification system of Thompson et al. (1997): transient (kochia), short-term persistent (giant foxtail), and long-term persistent (common lambsquarters, field pennycress, velvetleaf, and yellow foxtail). Of the two study species that also appear in the seedbank persistence database of Thompson et al. (1997), common lambsquarters and field pennycress, both received the same classification within this study as within the database.

Table 1. Half-lives of weed seeds in the soil seedbank estimated from published studies of long-term seedbank persistence, expressing seedling emergence from buried seed pools as a negative exponential function of burial time in years.[a]

Weed species	k[a]	$t_{0.5}$	R^2	Literature source
		yr		
Common lambsquarters	0.028	24.8	0.17	Burnside et al. 1996
Field pennycress	0.199	3.5	0.47	Burnside et al. 1996
Giant foxtail	0.906	0.80	0.99	Buhler and Hartzler 2001
Kochia	4.56	0.152	0.99	Burnside et al. 1996
Velvetleaf	0.083	8.3	0.81	Lueschen et al. 1993
Yellow foxtail	0.153	4.5	0.96	Burnside et al. 1996

[a] Abbreviations: k = seed decay constant; $t_{0.5}$ = predicted half-life of seeds in the soil seedbank.

The strong inverse relationship observed between short-term mortality of undamaged seeds in the 2-mo soil incubation and literature-based estimates of seed half-life in the soil seedbank ($t_{0.5}$) suggests that the estimated value of $t_{0.5}$ was a reasonable proxy for a direct measurement of $t_{0.5}$ for the seed accessions used in this study. Considerable variation observed in both short-term seed persistence and estimated $t_{0.5}$ indicated that the experimental design objective of achieving representation of species in different seed persistence classes was met.

Seed Chemical Protection. Twenty-five underivatized and twenty derivatized phenolic compounds were detected by GC/MS in the weed seed extracts (Table 2). Concentrations of phenolics varied by as much as four orders of magnitude among species. Taken as a class, seed concentrations of phenolics decreased exponentially in relation to seed $t_{0.5}$ from long-term burial studies ($y = 84 \cdot e^{(-0.044 \cdot t_{0.5})}$, $R^2 = 0.62$, $P < 0.05$) and were negatively associated with short-term persistence of undamaged seeds ($r = -0.90$, $P = 0.01$). Of the 45 phenolic compounds detected, only one, 2,4,6-tris(1,1,-dimethoxy)-phenol, showed a significant correlation by itself to seed $t_{0.5}$ ($r = -0.91$, $P < 0.05$). Seed concentrations of this compound across species were related to seed $t_{0.5}$ by a negative exponential relationship ($y = 51 \cdot e^{(-0.024 \cdot t_{0.5})}$, $R^2 = 0.84$, $P < 0.05$).

Within the broad class of phenols, *ortho*-dihydroxyphenols have previously been associated with seed persistence in the soil seedbank (Hendry et al. 1994). In this experiment, seed content of *ortho*-dihydroxyphenols, as determined by colorimetric methods, varied more than threefold between the least protected species (common lambsquarters, 9.2 μg g seed^{-1}) and the most protected species (kochia, 34.1 μg g seed^{-1}). The level of seed chemical protection from *ortho*-dihydroxyphenols declined exponentially as a function of $t_{0.5}$ estimated from long-term burial studies (Figure 3). Corroborating evidence for this relationship was provided by two additional sources of evidence. First, direct measurements of seed *ortho*-dihydroxyphenols as a class (including catechol, 4-methylcatechol, protocatechuic acid, and caffeic acid) by GC/MS were strongly correlated with the concentrations indicated by the colorimetric assay ($r = 0.80$, $P < 0.05$). These results offer independent verification of the reliability of the colorimetric assay of Hendry et al. (1994) for estimating total seed *ortho*-dihydroxyphenol content. Second, there was a strong negative association between short-term persistence of undamaged seeds in the mechanical damage bioassay (Figure 2) and seed *ortho*-dihydroxyphenol content by both

Table 2. Phenolic compounds detected by GC/MS in methanol extracts of seed homogenate for six weed species collected in Urbana, IL.

Compound	Common name	Weed species					
		ABUTH[a]	CHEAL	KOCSC	SETFA	SETLU	THLAR
		μg g^{-1} seed					
Underivatized							
1,2-Benzenediol	catechol	12.2 (2.3)	2.3 (0.3)	43.5 (5.9)	17.5 (1)	2.8 (0.1)	15.3 (0.9)
1,2-Benzenediol, 4-methyl-	4-methyl-catechol	11.1 (1.5)	0.0 (0)	7.4 (0.4)	2.6 (0)	4.1 (0.1)	0.0 (0)
1,4-Benzenediol, 2-methoxy-	2-methoxy-hydroquinone	6.8 (0.1)	1.2 (0.1)	9.1 (0.8)	6.7 (0.8)	6.2 (0.8)	1.3 (0.2)
1,4-Benzenediol/1,3-Benzenediol	hydroquinone/resorcinol	11.5 (1.9)	3.3 (0.3)	7.8 (0.1)	11.9 (1.1)	15.4 (1)	16.4 (1.3)
2-Methoxy-4-vinylphenol	NA	11.7 (1.1)	6.7 (0.5)	200.4 (16.8)	59.9 (5)	135.9 (29.9)	12.8 (1.7)
3,5-Dihydroxytoluene	orcin	6.3 (0)	0.7 (0.1)	6.9 (0.9)	3.3 (0.4)	2.5 (0.1)	0.1 (0)
3,5-Dimethoxyacetophenone	NA	7.2 (1.1)	3.3 (0.4)	17.1 (2.1)	28.3 (7.5)	45.1 (13)	467.4 (0.3)
Benzaldehyde	NA	0.7 (0.1)	0.3 (0.1)	0.0 (0)	0.0 (0)	0.0 (0)	0.7 (0)
Benzaldehyde, 2-methyl-	NA	0.0 (0)	11.4 (1.4)	0.0 (0)	2.7 (0.1)	0.0 (0)	3 (0.3)
Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	3,5-dimethoxy-vanillin	0.5 (0)	0.0 (0)	0.0 (0)	2.4 (0.1)	5 (1)	3.4 (0.2)
Benzaldehyde, 4-hydroxy-3-methoxy-	vanillin	2.8 (0.1)	2.2 (0.2)	8.9 (1.3)	10.6 (0.5)	11.3 (2.5)	0.0 (0)
Benzene, 1,2,3-trimethoxy-5-methyl-	NA	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	4.2 (0.1)	9.6 (1.8)
Benzene, 1,2,3-trimethoxy-5-methyl/-5-tert-Butylpyrogallol	NA	1.2 (0.2)	0.5 (0)	3.6 (0.2)	3.3 (0.4)	2.9 (0.1)	0.0 (0)
Benzenecarboxylic acid	benzoic acid	26.4 (3)	0.5 (0)	27.6 (1.8)	2.7 (0.1)	1.3 (0.5)	2.4 (0.2)
Benzofuran, 2,3-dihydro-	NA	6.2 (4.8)	1.9 (0.1)	25.7 (0.8)	17.1 (1.8)	28.6 (2.6)	7 (0.6)
Butyrophenone, 2,6-dihydroxy-4-methoxy-	desaspidinol	0.0 (0)	0.0 (0)	1.2 (0.2)	2.4 (0)	7.5 (0.1)	2.1 (0.2)
Ethanone, 1-(4-hydroxy-3-methoxyphenyl)-	acetovanillone	0.0 (0)	0.0 (0)	3.7 (0.4)	2.4 (0.1)	5.8 (0.1)	0.0 (0)
Indole	NA	1.0 (0)	4.0 (0.2)	2.6 (0.4)	1.8 (0.1)	1.0 (0.2)	2.1 (0.2)
Phenol	NA	15.3 (1.3)	7.2 (1.5)	15.5 (0.9)	18.3 (2.2)	10.4 (1.3)	11.9 (1.4)
Phenol, 2,6-dimethoxy-/Phenol, 3,4-dimethoxy-	NA	16.2 (0.4)	1.6 (0.3)	8.9 (0.9)	20.6 (2.3)	33.5 (4.8)	49.1 (0.7)
Phenol, 2,6-dimethoxy-4-(2-propenyl)-	NA	5.3 (0.6)	0.2 (0)	4 (0.3)	6.2 (0.7)	8.5 (2)	2.4 (0.1)
Phenol, 2-methoxy-	guaicol	8.9 (0.7)	39.7 (2.7)	42.3 (6.3)	21.3 (1.6)	48.3 (8.9)	41.1 (10.7)
Phenol, 2-methoxy-4-(1-propenyl)-	4-(1-propenyl)-guaicol	0.0 (0)	0.0 (0)	0.0 (0)	2.5 (0.2)	6.7 (0)	5.7 (0.4)
Phenol, 4-ethyl-2-methoxy-	4-ethyl-guaicol	0.4 (0)	0.0 (0)	25 (2.3)	1.8 (0.3)	1.4 (0.1)	1.1 (0.1)
Phenol, 4-methoxy-	NA	0.0 (0)	4.2 (0.1)	7.6 (0.1)	0.0 (0)	0.0 (0)	0.0 (0)
Derivatized[b]							
2-Hydroxy-2-phenylacetic acid	mandelic acid	28.1 (3.6)	0.0 (0)	1.2 (0.1)	3.8 (0.3)	1.3 (0.1)	1.1 (0.3)
3-(3,4-dihydroxyphenyl)-2-propenoic acid	caffeic acid	7 (0.9)	4.6 (0.5)	5.7 (0.1)	7 (1.8)	4.7 (0.1)	0.0 (0)
3-(4-hydroxy-3,5-dimethoxyphenyl)-prop-2-enoic acid	sinapic acid	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	1.1 (0.3)	6.9 (1.5)
3-(4-hydroxy-3-methoxy-phenyl)-prop-2-enoic acid	ferulic acid	4.1 (0.2)	11.1 (3.1)	39.2 (3)	10.7 (1.9)	15.2 (0.5)	0.0 (0)
3-(4-hydroxyphenyl)-2-propenoic acid	p-coumaric acid	2.9 (0.2)	13.2 (1.2)	2 (0.2)	8.8 (0.2)	17.1 (1.9)	6.2 (0.4)
3,4,5-Trihydroxy-1-cyclohexene carboxylic acid	shikimic acid	16.8 (7.7)	6.4 (0.9)	0.0 (0)	7.9 (1.5)	6.1 (1)	0.0 (0)
3-phenyl-2-propenoic acid	cinnamic acid	19.9 (1.4)	0.0 (0)	0.0 (0)	124.5 (34.7)	14.3 (3.7)	0.0 (0)
3-phenylpropanoic acid	hydrocinnamic acid	36.6 (6.1)	3.1 (0.5)	29.2 (5.8)	0.0 (0)	0.0 (0)	0.0 (0)
Benzenecarboxylic acid	benzoic acid	5.6 (1)	13.4 (2.8)	12.2 (17.3)	2.7 (3.8)	0.0 (0)	4.3 (6.1)
Benzoic acid, 2,5-dihydroxy-	gentisic acid	30.9 (8.3)	31.4 (7.1)	3.4 (0.4)	11.3 (1.9)	3.7 (0.2)	0.8 (0.2)
Benzoic acid, 3,4,5-trihydroxy-	gallic acid	14.9 (1.3)	0.0 (0)	0.4 (0.1)	0.0 (0)	0.0 (0)	0.0 (0)
Benzoic acid, 3,4-dihydroxy-	protocatechuic acid	14.7 (2.2)	30.9 (1.8)	24.6 (2.3)	14.9 (1.7)	9.1 (1.1)	18.4 (0.9)
Benzoic acid, 3,5-dimethoxy-4-hydroxy-	syringic acid	2.6 (0.1)	0.0 (0)	3.4 (0.2)	6.8 (0.7)	2.4 (0.1)	1 (0.1)
Benzoic acid, 3-hydroxy-	m-hydroxybenzoic acid	12.1 (1.3)	16.4 (0.5)	23.7 (0.5)	22.1 (2.5)	13.5 (0.4)	13 (0.6)
Benzoic acid, 3-methoxy-4-hydroxy-	vanillic acid	9 (0.2)	63.7 (8.5)	33 (5.6)	27.6 (2.6)	36.9 (6.3)	15.1 (0.1)
Benzoic acid, 4-hydroxy-	p-hydroxybenzoic acid	63.9 (4.6)	41.6 (2.3)	29.1 (0.5)	32 (4.1)	39.5 (2.1)	30.5 (2)
Cinnamyl alcohol, 4-hydroxy-3,5-dimethoxy-	sinapyl alcohol	0.0 (0)	0.0 (0)	0.0 (0)	8 (0.9)	6 (1.5)	0.0 (0)
Ethanol, 3,4-dihydroxyphenyl-	NA	0.0 (0)	0.0 (0)	316.5 (15.2)	4 (0.4)	0.0 (0)	0.0 (0)
Phenol, 2,4,6-tris(1,1-dimethylethyl)-	NA	39.4 (1.5)	29 (5.5)	56.4 (5.6)	45 (6.1)	47.8 (2)	46.1 (8.4)
Quinic acid, 3-cis-(4,5-dihydroxyphenyl propanal)	3-cis-caffeoylquinic acid	31.7 (5.8)	0.0 (0)	0.0 (0)	31.4 (0.2)	6 (0.6)	0.0 (0)

[a] Bayer code explanations: ABUTH = velvetleaf (*Abutilon theophrasti*), CHEAL = common lambsquarters (*Chenopodium album*), KOCSC = kochia (*Kochia scoparia*), SETFA = giant foxtail (*Setaria faberi*), SETLU = yellow foxtail (*Setaria glauca*), THLAR = field pennycress (*Thlaspi arvense*).

[b] To increase volatility, hard-to-detect phenolic compounds were derivatized with N-Methyl-N-(trimethylsilyl) trifluoroacetamide.

colorimetric ($r = -0.82$, $P < 0.05$) and GM/MS ($r = -0.88$, $P < 0.05$) methods.

Contrary to our hypothesis of increasing seed chemical protection with increasing seed persistence in the soil seedbank, seed concentrations of both total phenolic compounds and *ortho*-dihydroxyphenols declined in relation to increases in both short- and long-term seedbank persistence of the six weed species included in this study. This finding contrasts with those of other studies relating seedbank persistence and seed chemical defense levels (Hendry et al. 1994; Veldman et al. 2007), in which chemical defense was associated with seedbank persistence. It is possible that other seed defense compounds not measured here, such as cyanoglycosides (Hendry et al. 1994) or phytoalexins (Halloin 1983) might have increased in proportion to seedbank persistence levels. Although this question merits further empirical testing for seeds of weed species, it should be noted that phenols found in weed seeds have been found to have suppressive effects on seed-borne fungi (Paszkowski and Kremer 1988), and that cyanoglycoside concentrations were shown to be unrelated to seedbank persistence (Hendry et al. 1994).

Alternatively, this discrepancy in results might stem from differences in the characteristics of the populations of plants being examined in the various studies. Hendry et al. (1994) related concentrations of *ortho*-dihydroxyphenols and cyanoglycosides to seedbank persistence for 81 British isle species from diverse habitats, with the species evenly split between

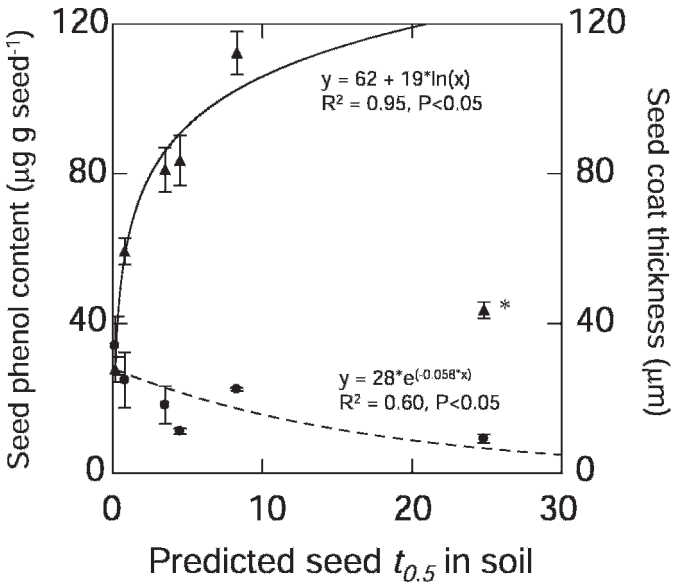

Figure 3. Seed *ortho*-dihydroxyphenol content (dashed line, circles) and seed coat thickness (solid line, triangles) varied as negative exponential and natural logarithm functions, respectively, with seed half-life ($t_{0.5}$) in the soil seedbank estimated from literature values. The regression between seed coat thickness and $t_{0.5}$ excluded the common lambsquarters datum (*; studentized residual = −5.5).

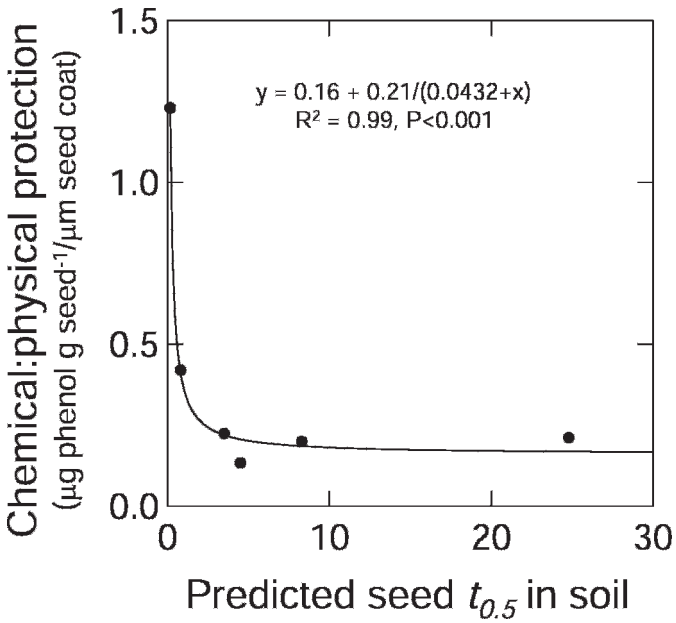

Figure 4. The importance of seed chemical protection, relative to physical protection, decreased hyperbolically as seed persistence in the soil seedbank increased.

transient and persistent seedbanks (Thompson et al. 1997). Cyanoglycoside concentrations were unrelated to seedbank persistence for these species. Whereas Hendry et al. (1994) observed wide, overlapping ranges for seed *ortho*-dihydroxyphenol concentrations of transient and persistent seed classes (0.29 to 162 and 3.7 to 557 μg g seed^{-1}, respectively), the observed range of seed *ortho*-dihydroxyphenol concentrations in our study, as determined via the same colorimetric method, was much narrower (9 to 34 μg g seed^{-1}). Moreover, seedbank persistence was treated as a continuous, rather than binary, variable. It might be that, by selecting species found only within one highly specialized ecosystem and locale (grain crop production fields in the midwest United States), we observed a relationship between chemical defense and seedbank persistence closely tied to the abiotic and biotic conditions of that environment.

Seed Physical Protection. Seed physical protection, as represented by seed coat thickness, varied more than fourfold between the least protected species (kochia, 27.7 μm) and the most protected species (velvetleaf, 112 μm) (Figure 3). When the association between seed physical protection and $t_{0.5}$ was examined across all study species, there was no significant relationship ($r = -0.09$, NS). Analysis of residuals indicated that the common lambsquarters datum was an outlier (studentized residual = −5.5) from the rest of the study species. Seed coat thickness was positively associated with seed mass ($r = 0.81$, $P < 0.05$), hence common lambsquarters, the lightest of the study species (and also the most persistent in the soil seedbank), had a thinner seed coat than other study species, with the exception of kochia. In proportion to its mass, however, common lambsquarters had a thicker seed coat than other study species (data not shown). When common lambsquarters was excluded from the analysis, seed coat thickness was found to increase as a function of the natural logarithm of $t_{0.5}$ (Figure 3). Seed coat thickness was unrelated to short-term seed persistence in soil, and there was no indication of outliers in this analysis. Over the range of seed half-lives studied, seed chemical protection, relative to physical protection, decreased with increasing $t_{0.5}$ for both *ortho*-dihydroxyphenols (Figure 4) and phenols as a class ($y = 7 + (0.26/(-0.07 \cdot t_{0.5}))$; $R^2 = 0.98$, $P < 0.05$).

Consequences of Mechanical Damage. Mechanical damage to the weed seed coat increased mortality during soil burial for all six of the study species (main effect of damage: $F_{1, 219} = 186$, $P < 0.0001$; Figure 2), and increases in mortality due to damage varied by species (species by damage interaction: $F_{5, 219} = 1374$, $P < 0.0001$). Seed mortality due to different damage classes did not vary for common lambsquarters, field pennycress, and velvetleaf. In contrast, μ was greater for the ground and sliced treatments than the pierced treatment for kochia, and μ was greatest in the ground treatment, intermediate in the pierced treatment, and lowest in the sliced treatment for giant and yellow foxtail.

The relative effects of the different damage treatments on seed mortality, once mortality levels in the undamaged control were factored out, varied with seed characteristics of the study species (Table 3). Seed mortality due to the piercing treatment was positively correlated with $t_{0.5}$, negatively correlated with seed concentrations of *ortho*-dihydroxyphenols and total phenols, and positively correlated with seed coat thickness, whereas μ due to the slicing and grinding treatments were unrelated to these seed characteristics. The grinding treatment resulted, as expected, in mortality rates greater than 70% for all six species, with nearly complete seed mortality in giant foxtail, kochia, and velvetleaf. Seed mass and seed coat thickness were positively correlated with μ due to grinding, but did not have significant associations with μ due to the other mechanical damage treatments.

Physical seed protection was a critical persistence mechanism for all six weed species in this study, as revealed by direct measurements of seed coat thickness and indirect measurements via mechanical damage to the seed coat. Direct

Table 3. Pearson correlations between seed characteristics and seed mortality during soil burial due to mechanical damage to seed coat.

Mechanical damage type	Predicted seed $t_{0.5}$ in soil	Seed *o*-dhp concentration[a]	Seed total phenols[b]	Seed mass	Seed coat thickness
Piercing	0.42**	−0.35*	−0.43***	0.17 NS	0.36*
Slicing	0.06 NS	0.13 NS	−0.04 NS	0.24 NS	0.15 NS
Grinding	−0.02 NS	−0.07 NS	−0.12 NS	0.43**	0.40**

[a] Explanation of abbreviation: *o*-dhp = *ortho*-dihydroxyphenols, determined colorimetrically.
[b] Total phenols, determined by gas chromatography–mass spectrometry.
NS indicates nonsignificant effect. Bonferroni-corrected probabilities: * = $P < 0.05$, ** = $P < 0.01$.

measurements of seed coat thickness demonstrated an inverse relationship between chemical and physical seed protection, with an increasing importance of physical protection for longer-lived seeds. Mechanical damage bioassays corroborated this finding: chemical defense levels declined with increasing short and long-term seedbank persistence, but physical integrity of the seed coat was essential to seed survival during the 2-mo soil incubation for all six species. Other studies have linked physical seed protection (Mohamed-Yasseen et al. 1994; Rodgerson 1998) to seedbank persistence.

With the exception of the piercing treatment, seed chemical defenses did not mitigate seed decay due to mechanical damage treatments. Presumably, the slicing and grinding treatments removed a large enough portion of the seed that opportunistic fungi or bacteria were able to overwhelm seed chemical defenses. In the piercing treatment, however, seed mortality declined with increasing concentrations of *ortho*-dihydroxyphenols and total phenolic compounds. Piercing damage of weed seeds commonly occurs due to predispersal seed feeding by arthropods with piercing-sucking mouthparts, and has been observed to increase weed seed decay due to fungal attack (Kremer and Spencer 1989). Therefore, although weed seed phenols don't appear to serve as the primary defense against decay, nor drive seedbank persistence, they might provide a secondary defense against superficial damage to the seed coat by granivores.

Management Implications

At present, there are few practical methods for reducing weed seedbank persistence (Gallandt 2006). One approach typical of commercial grain production 50 yr ago, which fell out of use as dependence on herbicides for weed control increased, and more recently has received renewed interest, is the collection and destruction of weed seeds by harvest machinery, either through chaff collection and milling (Leeson and Thomas 2001; Shirtliffe and Entz 2005) or by modifying harvest machinery to damage weed seeds as they are separated from the harvestable product (Slagell-Gossen et al. 1998). The results presented here offer strong support for weed seed destruction at harvest as a seedbank management tactic, because they identify a potential weakness in the way weed seed defenses are constructed: the most persistent seeds in the weed seedbank (e.g., common lambsquarters and velvetleaf) appear to have the weakest chemical defenses. In practical terms, this means that ''seed predator combines'' need not turn seeds into fine homogenates in order to be effective. Such a requirement would be a major obstacle to the development of such a device, because it would create conflicts between energy use and weight limitations, both of which are already subject to very fine tolerances in combine harvesters (Baruah and Panesar 2005). Instead, these data suggest that only small amounts of damage need be applied to each seed to ensure the rapid demise of the most recalcitrant species in the weed seedbank. A second design consideration introduced by the data is that grinding efficacy and seed mass are inversely related. The positive correlation between seed mass and μ due to grinding indicated that seeds with greater inertia were more susceptible to the 2 s pulse in the seed grinder than lighter seeds that were more easily moved about by the grinder blades. Hammer or roller mills, which trap seeds to be milled against an immovable surface, might therefore be a more effective tool for targeting the smallest seeds than grinding based on whirling blades, as used in this study.

Another approach to weed seedbank management that would appear to be supported by these data is to manage cropping systems for enhanced rates of predispersal seed predation. Despite the enormous quantities of weed seeds that can be consumed immediately following dispersal (Menalled et al. 2006), many seeds escape this fate through movement into deeper soil layers. Small, highly persistent seeds, such as common lambsquarters, are particularly likely to escape postdispersal predation via this mechanism (Thompson et al. 1993). Because these same seeds have been shown to decay rapidly once the seed coat is breached, predispersal predators would have two chances to increase seed mortality of these species: directly, by consuming the viable portion of the seed, or indirectly, by piercing the seed coat and making the interior of the seed readily accessible to microbial predators (Kremer and Spencer 1989). Studies of predispersal predation of weed seeds are less common than those of postdispersal predation, but suggest that cropping system management can have a significant impact on predispersal predation rates of species with long-lived seeds (DeSousa et al. 2003; McCarty and Lamp 1982; Nurse et al. 2003)

Demographic and long-term community studies indicate that weed seedbank management should be a central component of integrated weed management systems targeted at annual weeds (Davis 2006; Menalled et al. 2001). Making weed seedbank management tactics a reality will require a comprehensive research effort involving experts in complementary disciplines, including agricultural engineering, entomology, plant ecology, seed biology, and weed science.

Sources of Materials

[1] Benchtop precision mill, Wiley Mini-Mill, Thomas Scientific, Swedesboro, NJ 08085.

[2] Spectrophotometer, Spectronic 20, Carolina Biological Supply, Burlington, NC 27215.

[3] Zebron ZB-WAX *plus* column, Phenomenex, 411 Madrid Ave., Torrance, CA, 90501–1430.

[4] Agilent 6890N GC, an Agilent 5973 mass selective detector, and HP 7683B autosampler, Agilent Inc., 5301 Stevens Creek Blvd., Santa Clara, CA 95051.

[5] Electron impact mass spectra libraries: NIST05 and the Wiley Registry, 8th Edition, Palisade Corporation, 798 Cascadilla St., Ithaca, NY 14850.

[6] AMDIS program, National Institute for Standards and Technology, Gaithersburg, MD, 20879.

[7] Field-emission environmental scanning electron microscope; EDAX XL 30 ESEM-FG, FEI Company, Hillsboro, OR 97124.

[8] Sputter coating, Delton Desk II TSC, Denton Vacuum Co, Moorestown, NJ 08057.

[9] Image analysis software, ImagePro Plus, Media Cybernetics, Bethesda, MD 20814.

[10] Coffee grinder, KMM30, Braun Mfg. Co., South Boston, MA 02017.

[11] 125L Incubator, Conviron, Winnipeg, Canada RH3 OR9.

[12] SYSTAT Software, San Jose, CA 95110.

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